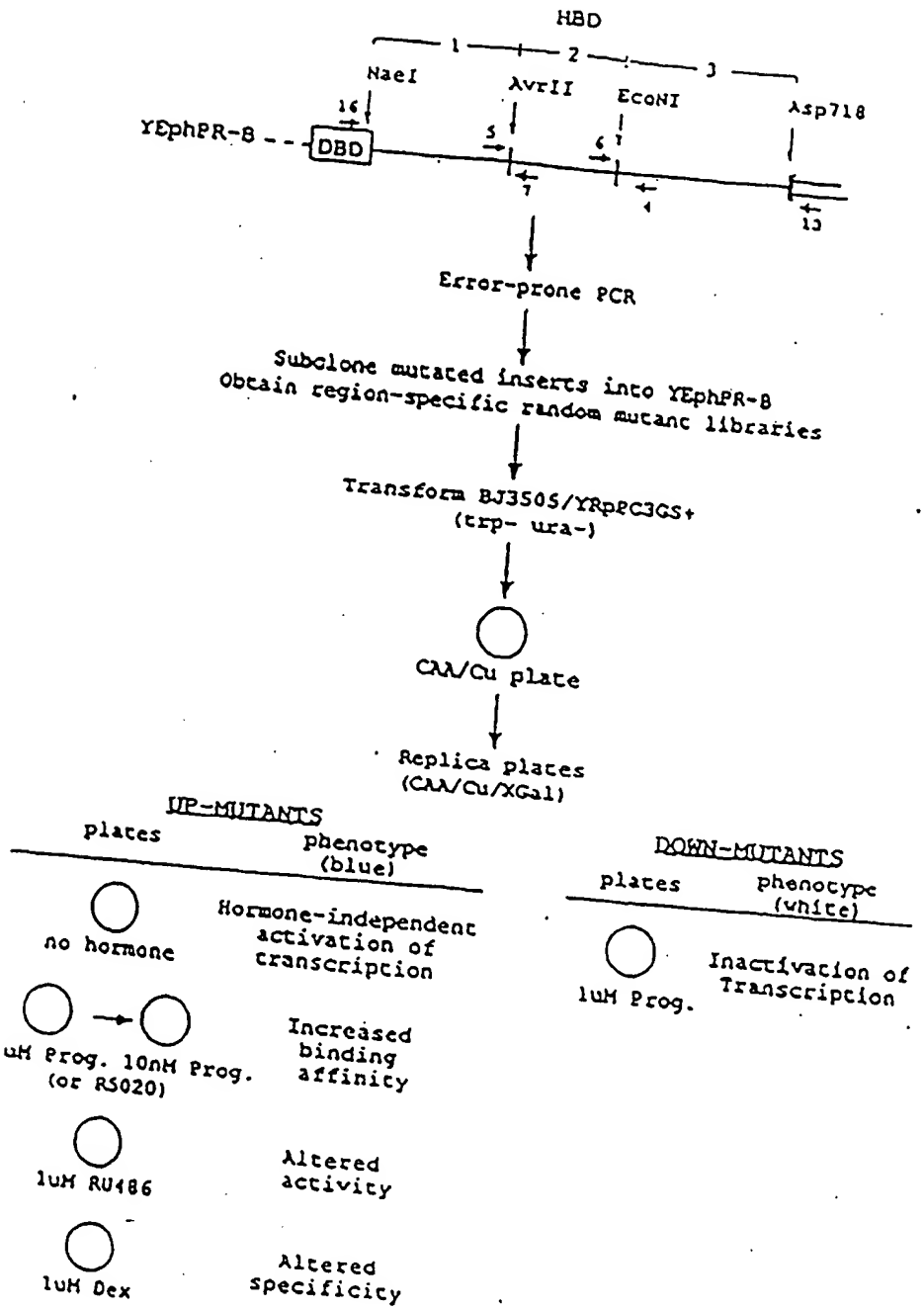

HBD
1
2
3
NaeI
AvrII
EcoNI
Asp718
16
YEphPR-B
DBD
5
6
7
4
13
Error-prone PCR
Subclone mutated inserts into YEphPR-B
Obtain region-specific random mutanc libraries
Transform BJ3505/YRpPC3GS+
(trp- ura-)
CAA/Cu plate
Replica plates
(CAA/Cu/XGal)
UP-MUTANTS
plates
phenotype
(blue)
no hormone
Hormone-independent
activation of
transcription
1uM Prog. 10nM Prog.
(or R5020)
Increased
binding
affinity
1uM RU486
Altered
activity
1uM Dex
Altered
specificity
DOWN-MUTANTS
plates
phenotype
(white)
1uM Prog.
Inactivation of
Transcription

Figure 1

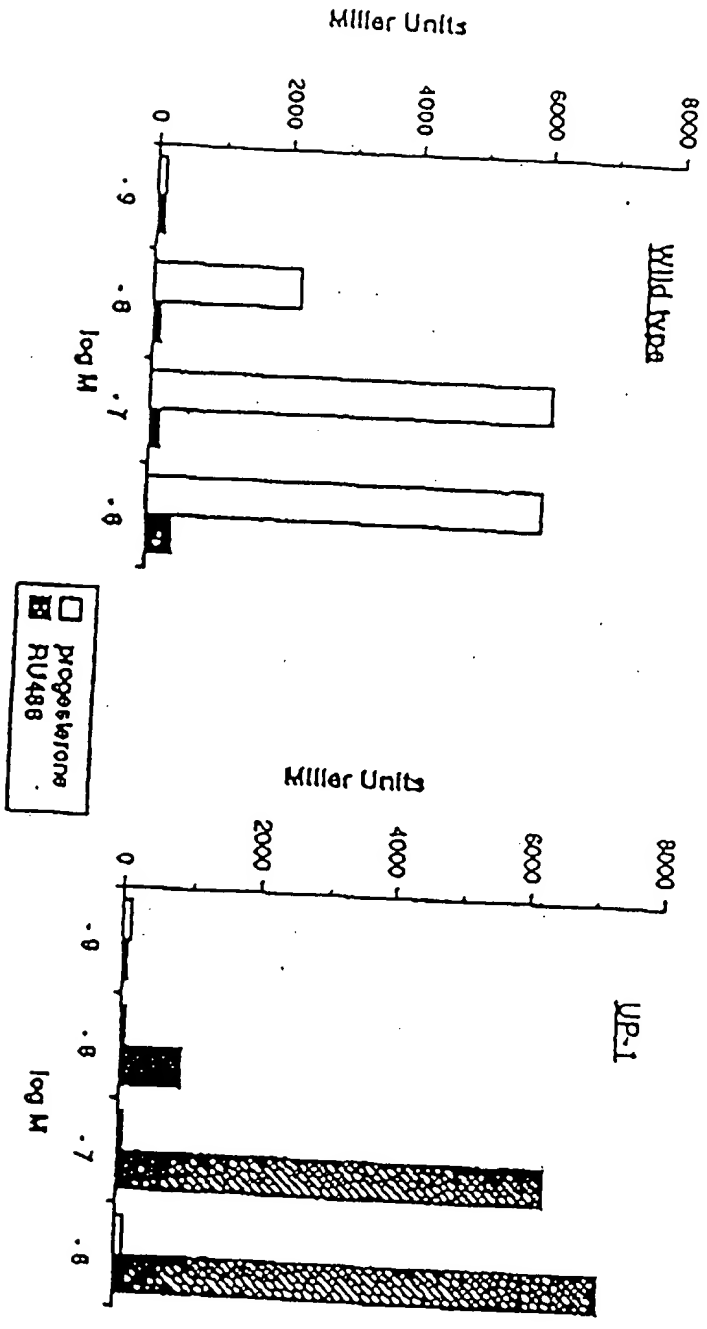

A
Miller Units
0
2000
4000
6000
8000
-6
-8
-7
-8
log M
Wild type
Progesterone
RU486
Miller Units
0
2000
4000
6000
8000
-6
-8
-7
-8
log M
UP-1

Figure 2A

B

DNA sequence:

2636
WT ..AAC TTG CAT GAT CTT GTC AAA CAA CTT CAT CTG TAC TGC TTG..

UP-1..AAT TGC ATG ATC TTG TCA AAC AAC TTC ATC TGT ACT GCT TGA

Protein sequence:

879
WT ..Asn Leu His Asp Leu Val Lys Gln Leu His Leu Tyr 891 Cys Leu..

UP-1..Asn Cys Met Ile Leu Ser Asn Asn Phe Ile Cys Thr Ala

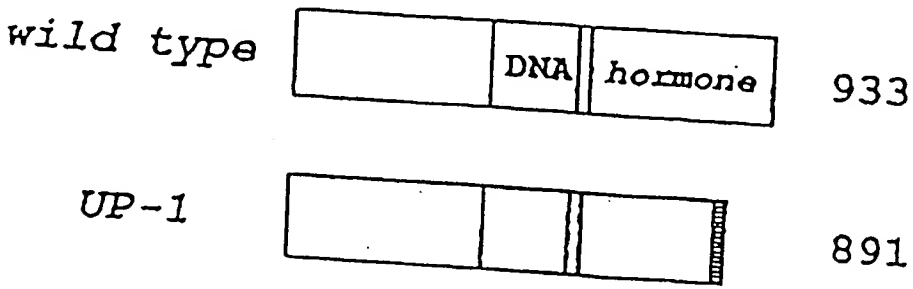


Figure 2B

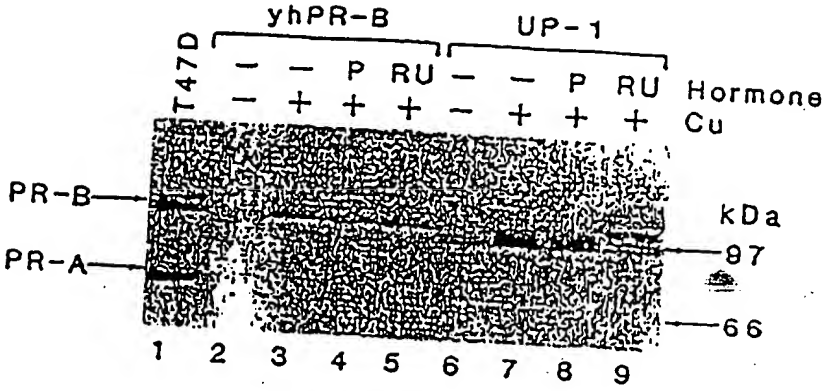


Figure 3

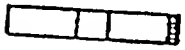
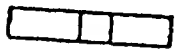
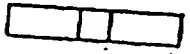
hPR Constructs		Transcriptional Activity (Miller Units)			Specific Binding (nM)	
		-	P	RU	P	RU
YEphPR-B (933)		86	6200	586	1.0	1.3
UP-1		286	466	8050	0.02	1.6
YEphPR-B879		166	242	5900	0.04	1.8
YEphPR-B891		243	226	6175	0.03	1.6

Figure 4

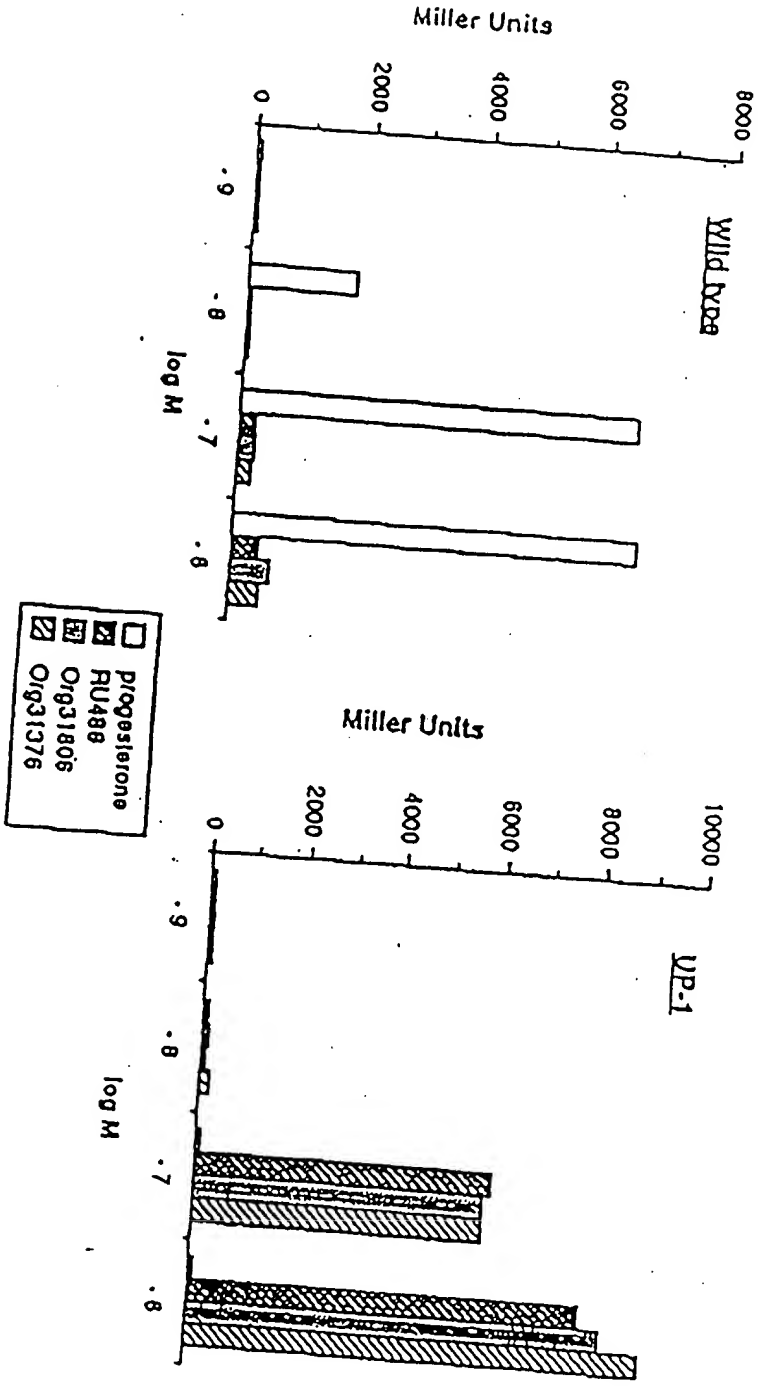

A
Miller Units
0
2000
4000
6000
8000
Wild type
log M
-6
-8
-7
-8
progesterone
RU486
Org31806
Org31376
Miller Units
0
2000
4000
8000
10000
UP-1
log M
-6
-8
-7
-8

Figure 5A

B

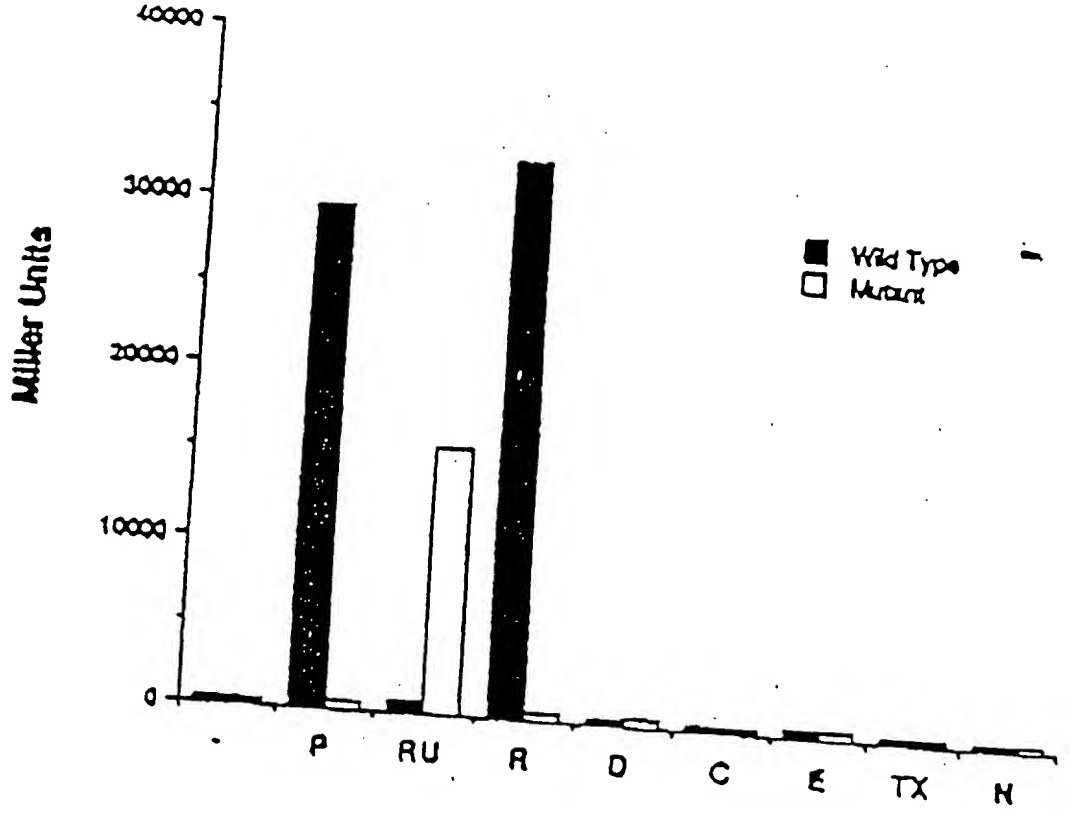

40000
30000
20000
10000
0
Miller Units
Wild Type
Mutant
P
RU
R
D
C
E
TX
N

Figure 5B

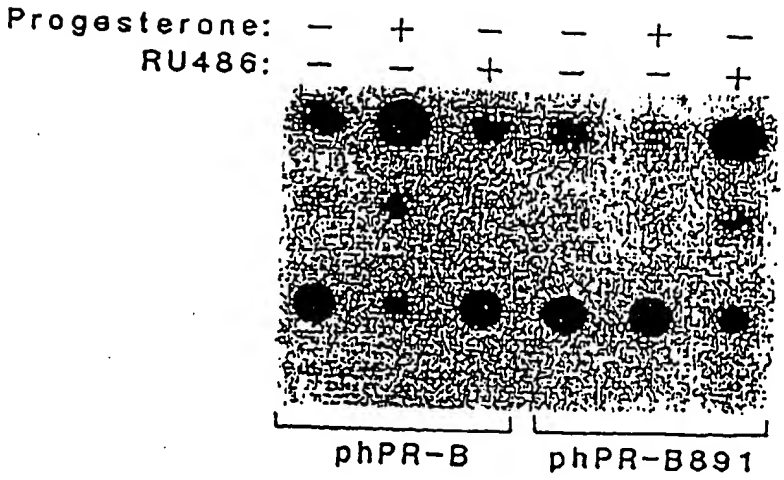


Figure 6A

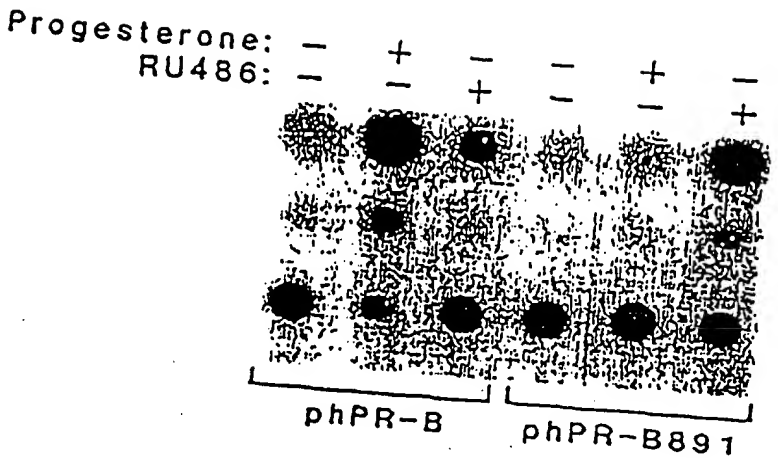


Figure 6B

GR-PR Fusion Constructs

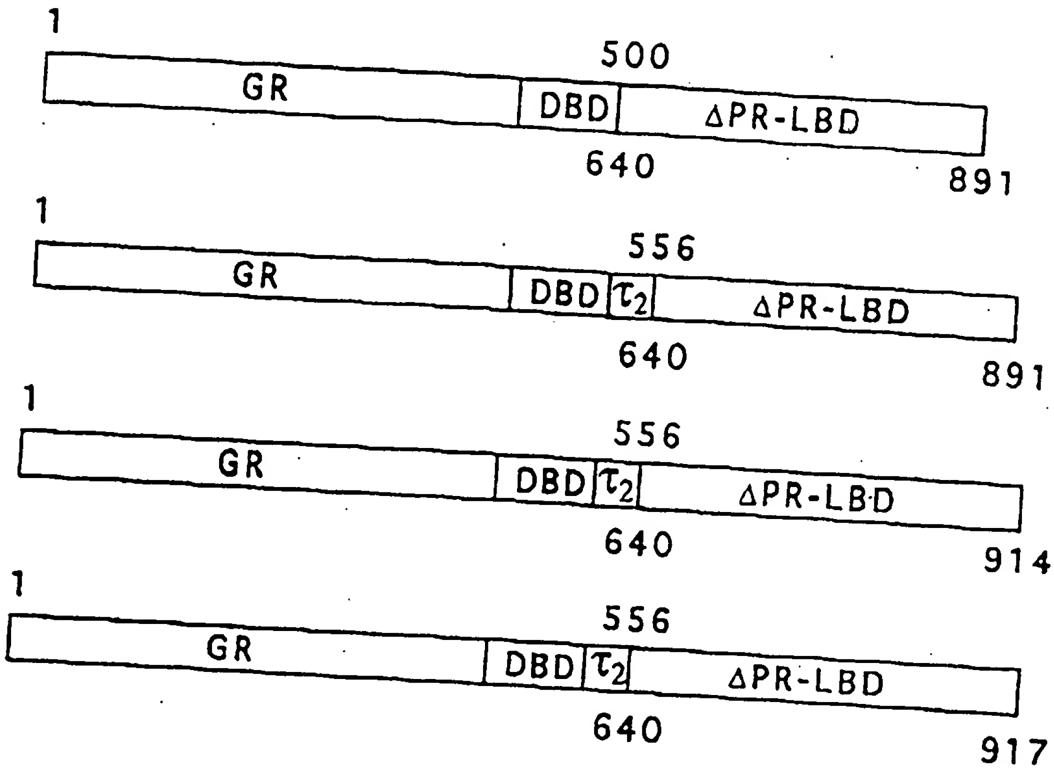
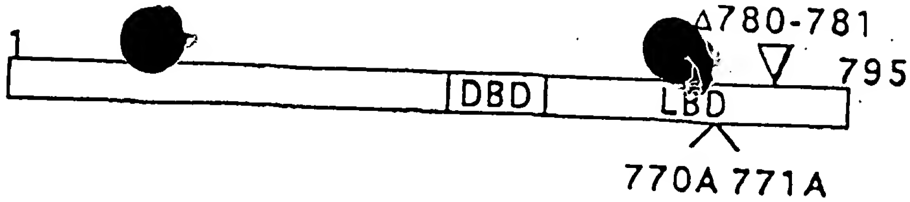


Figure 7



HUMAN GR MUTANTS

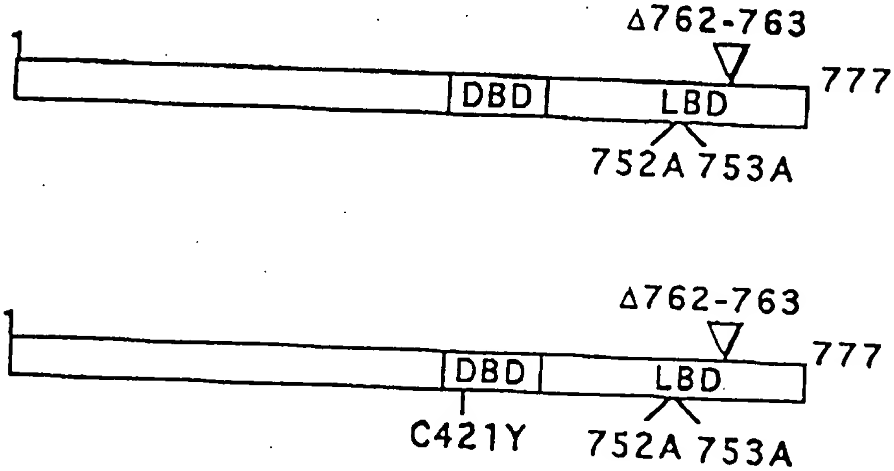


Figure 8

FIGURE 9

ORIGIN

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 121 aagttttcag ggtgttgttt agaatgggaa gatgtccctt gtatcaccat ggaccctcat
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 481 gaaacaacta catcctggtc atcatcctgc ctttctcttt atggttacaa tgatatacac
 541 tgtttgagat gaggataaaa tactctgagt ccaaaccggg cccctctgct aaccatgttc
 601 atgccttctt ctttttccta cagctcctgg gcaacgtgct ggttgttgtg ctgtctcatc
 661 atttggcaa agaattcact cctcaggtgc aggctgccta tcagaaggtg gtggctggtg
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 781 gggacatcat gaagcccctt gagcatctga cttctggcta ataaaggaaa tttattttca
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 901 atcatttaaa acatcagaat gagtatttgg tttagagttt ggcaacatat gccatatgct
 961 ggctgccatg aacaaaggtg gctataaaga ggtcatcagt atatgaaaca gcccctgct
1021 gtccattcct tattccatag aaaagccttg acttgaggtt agattttttt tatattttgt
1081 tttgtgttat tttttctttt aacatcccta aaattttcct tacatgtttt actagccaga
1141 tttttcctcc tctcctgact actcccagtc atagctgtcc ctcttctctt atgaactcga
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1561 ataacgcagg aaagaacatg tgagcaaaag gccagcaaaa ggccaggaac cgtaaaaagg
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1801 ttctcccttc gggaagcgtg gcgctttctc aatgctcacg ctgtaggtat ctcagttcgg
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1981 tggcagcagc cactggtaac aggattagca gagcgaggta tgtaggcggt gctacagagt
2041 tcttgaagtg gtggcctaac tacggctaca ctagaaggac agtatttggt atctgcgctc
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5761 atgtaagctc tcctccatcc agctcgtcag cagccacggg accacctccc aagctctgcc
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5881 aagtattctt taaaagagca gtggaaggac agcacaatta cctttgtgct ggaagaaacg
5941 attgcatcat tgataaaatt cgaaggaaaa actgcccagc atgccgctat cggaaatgtc
6001 ttcaggctgg aatgaacctt gaagctcgaa aaacaaagaa aaaaatcaaa gggattcagc
6061 aagccactgc aggagtctca caagacactt cggaaaatcc taacaaaaca atagttcctg
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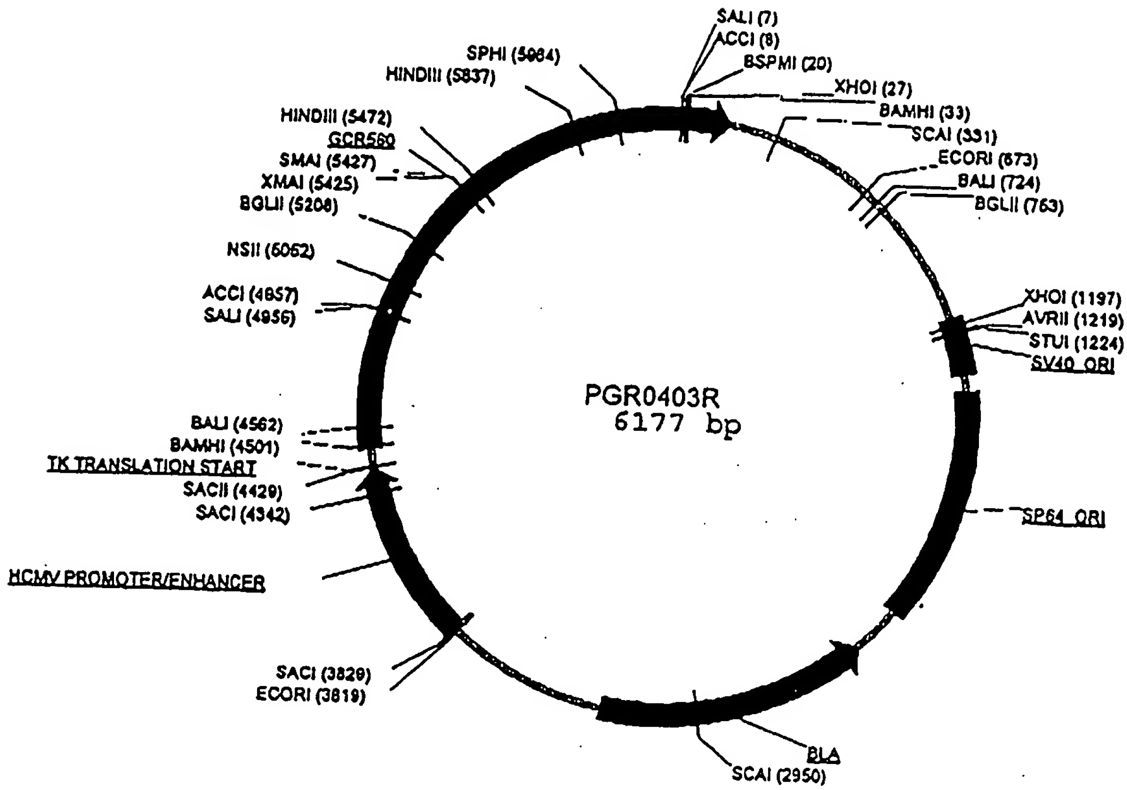
11

57

6178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6177 bp
SALI (7)
ACCI (8)
BSPMI (20)
XHOI (27)
BAMHI (33)
SCAI (331)
ECORI (673)
BALI (724)
BGLII (763)
XHOI (1197)
AVRII (1219)
STUI (1224)
SV40 ORI
SP64 ORI
BLA
SCAI (2950)
SACI (3829)
ECORI (3819)
HCMV PROMOTER/ENHANCER
SACI (4342)
SACII (4429)
TK TRANSLATION START
BAMHI (4501)
BALI (4562)
SALI (4956)
ACCI (4957)
NSII (5052)
BGLII (5208)
XMAI (5425)
SMAI (5427)
GCR560
HINDIII (5472)
HINDIII (5837)
SPHI (5984)

Figure 10

receptor	treatment	pg CAT protein induced	Normalized to control
wild-type hGR	control	0.4	1
	DEX	64.4	161
	RU	1.4	3.5
GRPR fusion	control	0.9	1
	DEX	0.6	0.7
	RU	5.4	6
wild-type rat GR	control	2.2	1
	DEX	26.4	12
	RU	6.3	2.9
CS1.CD	control	2.2	1
	DEX	1.8	0.8
	RU	29.6	13.5

Figure 11

Glucocorticomimetic Receptor

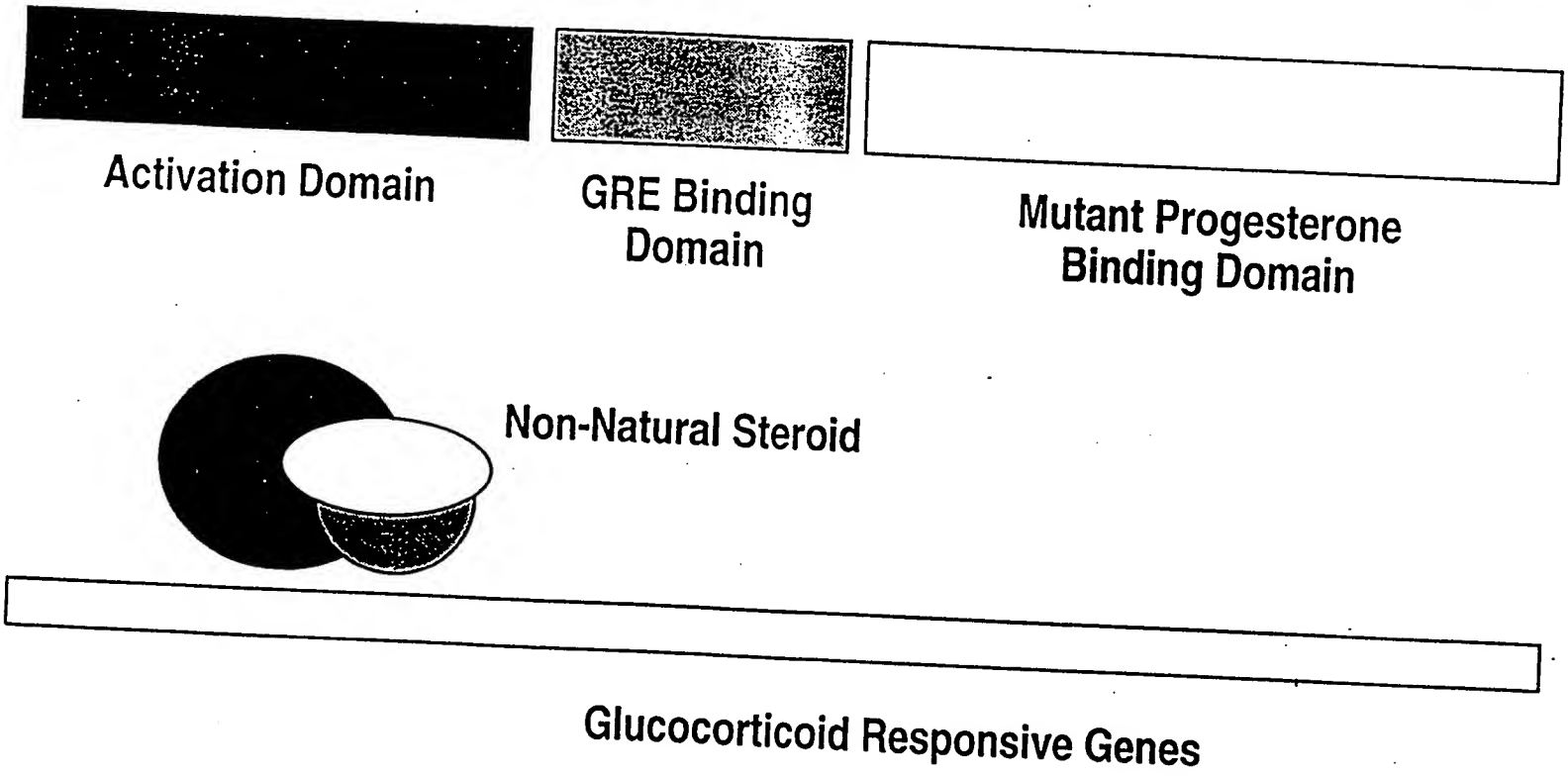

Figure 12

Gene Switch

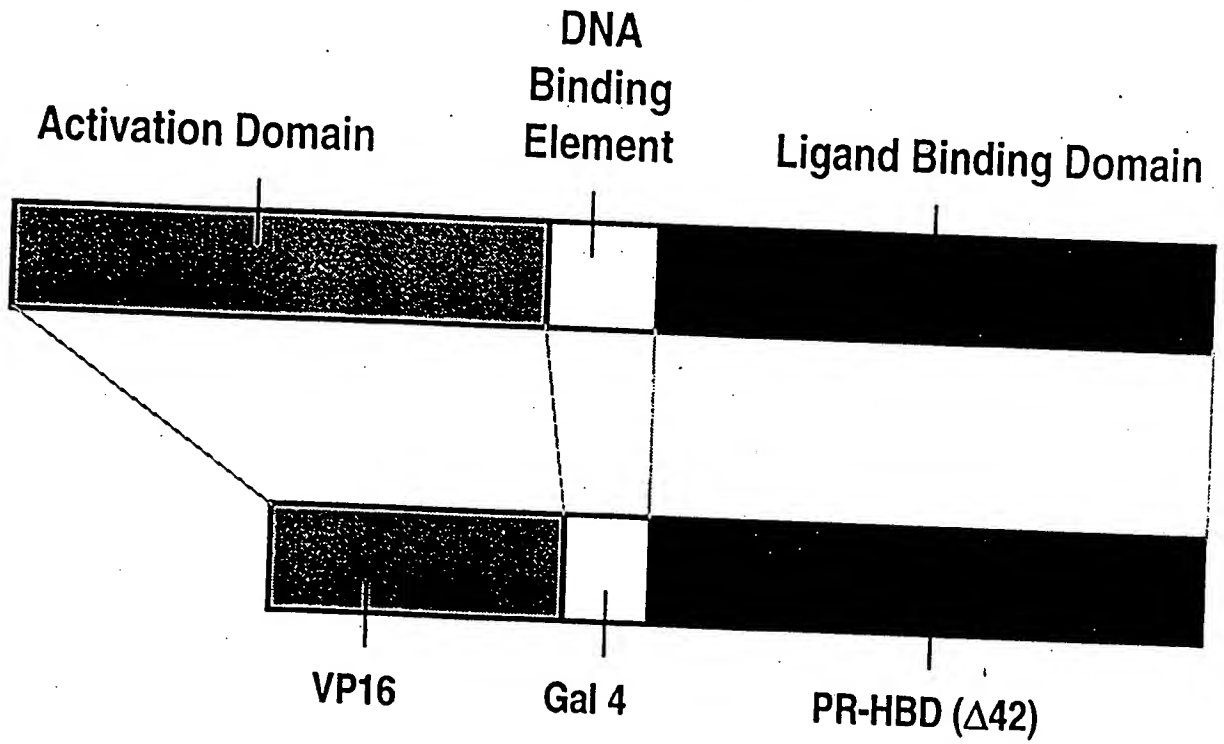

Figure 13

FIG. 14

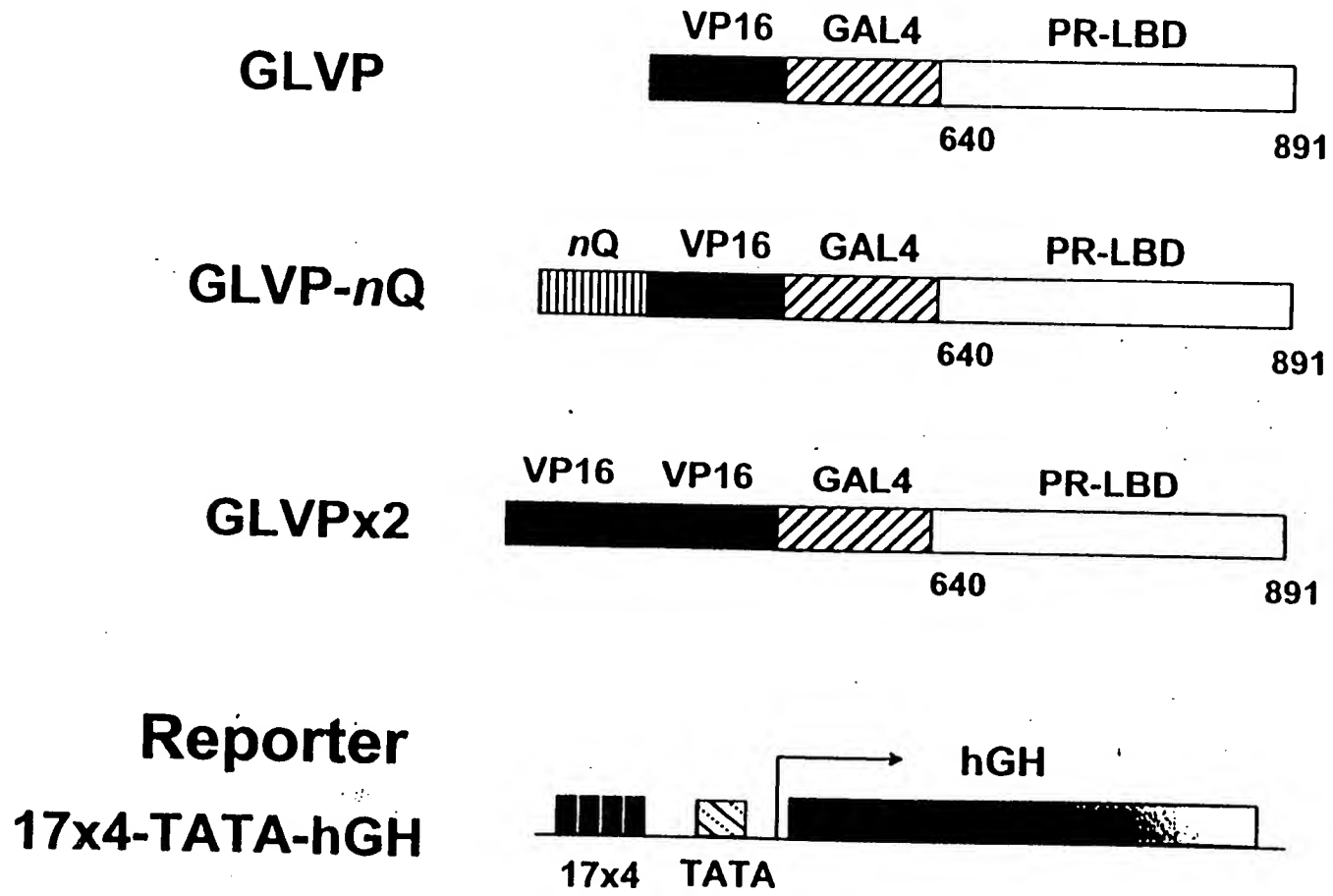

FIG. 15

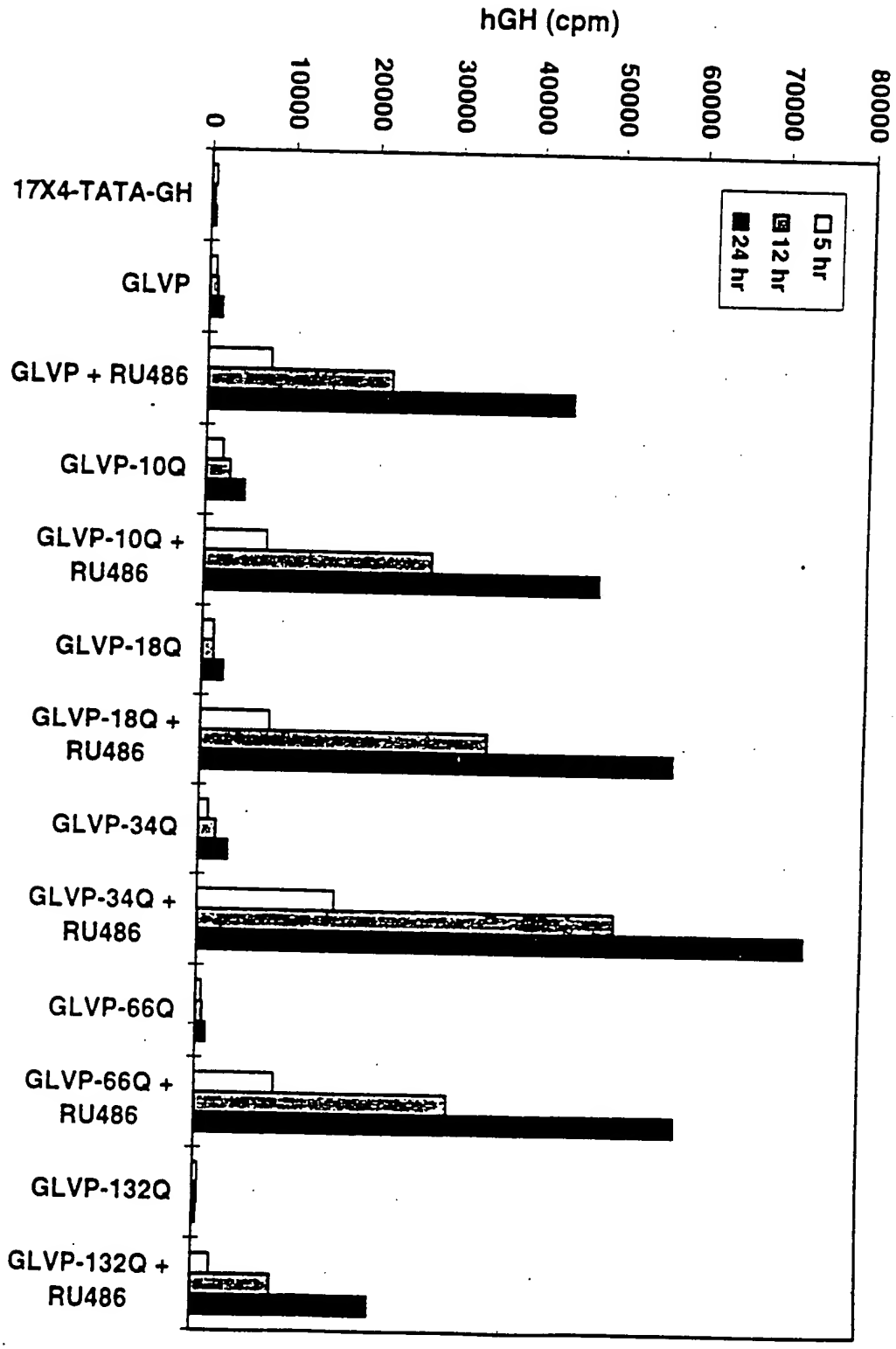

FIG. 16

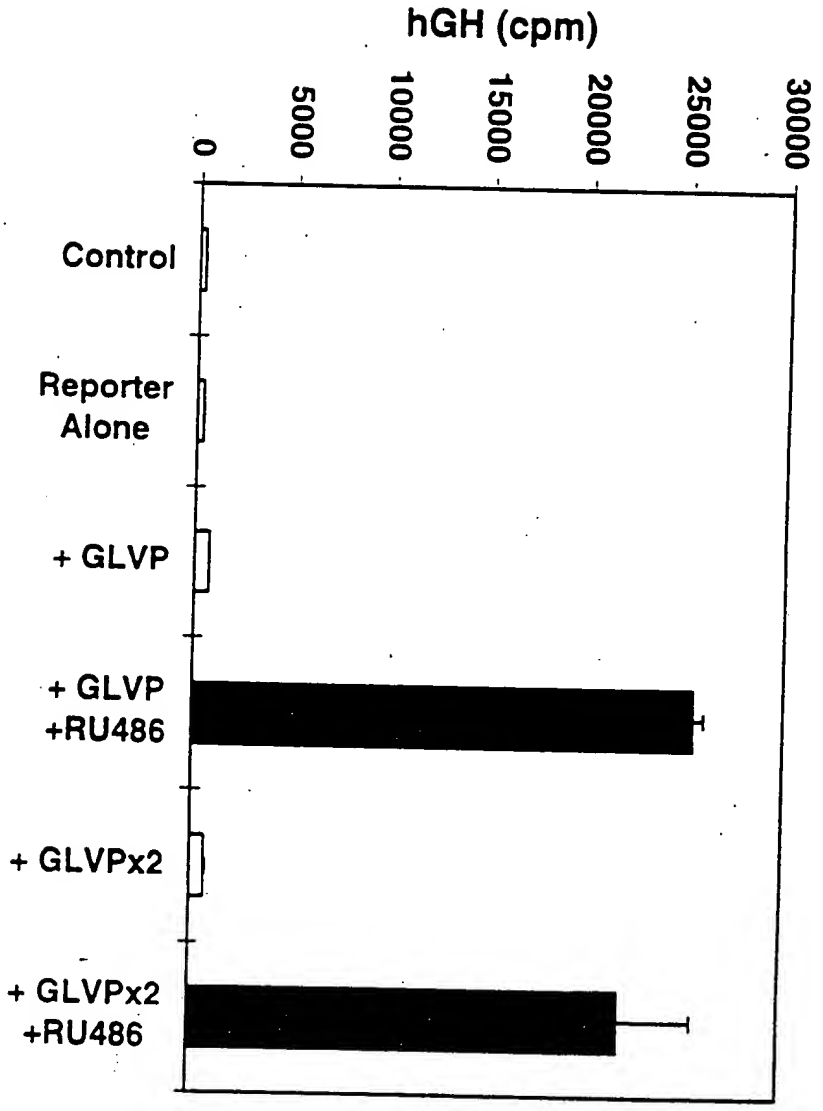
hGH (cpm)
0
5000
10000
15000
20000
25000
30000
Control
Reporter Alone
+ GLVP
+ GLVP +RU486
+ GLVPx2
+ GLVPx2 +RU486

FIG. 17

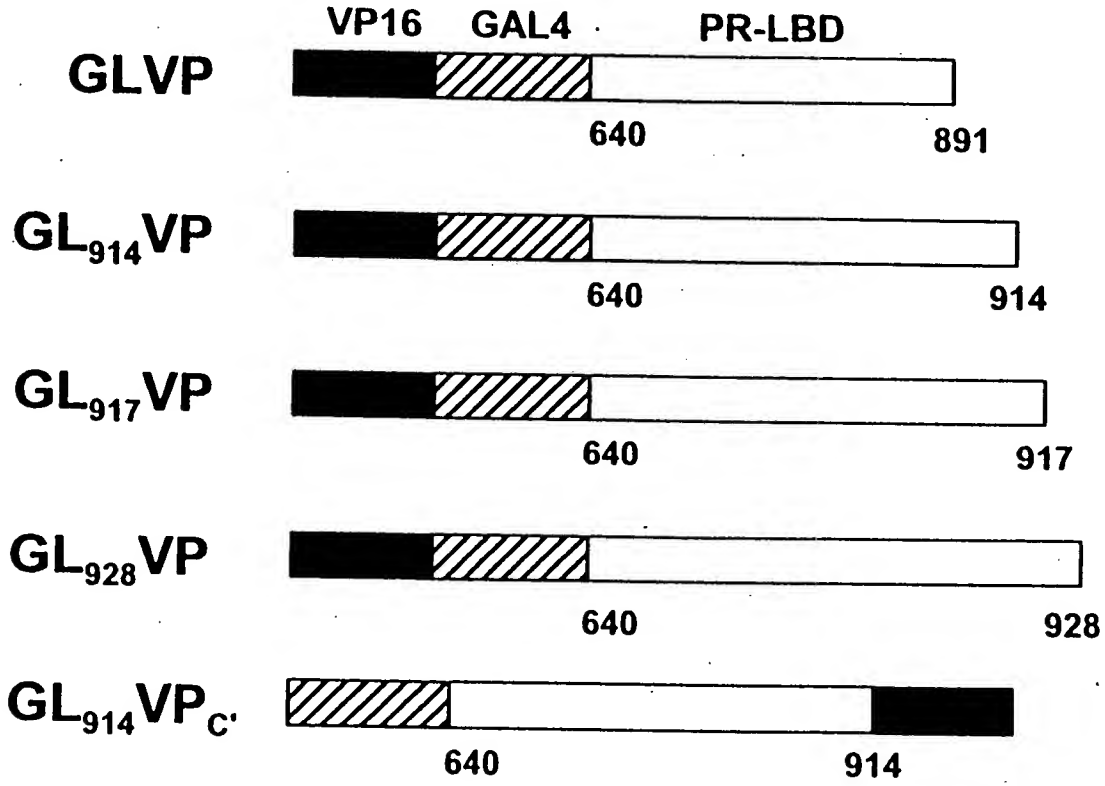

FIG. 18

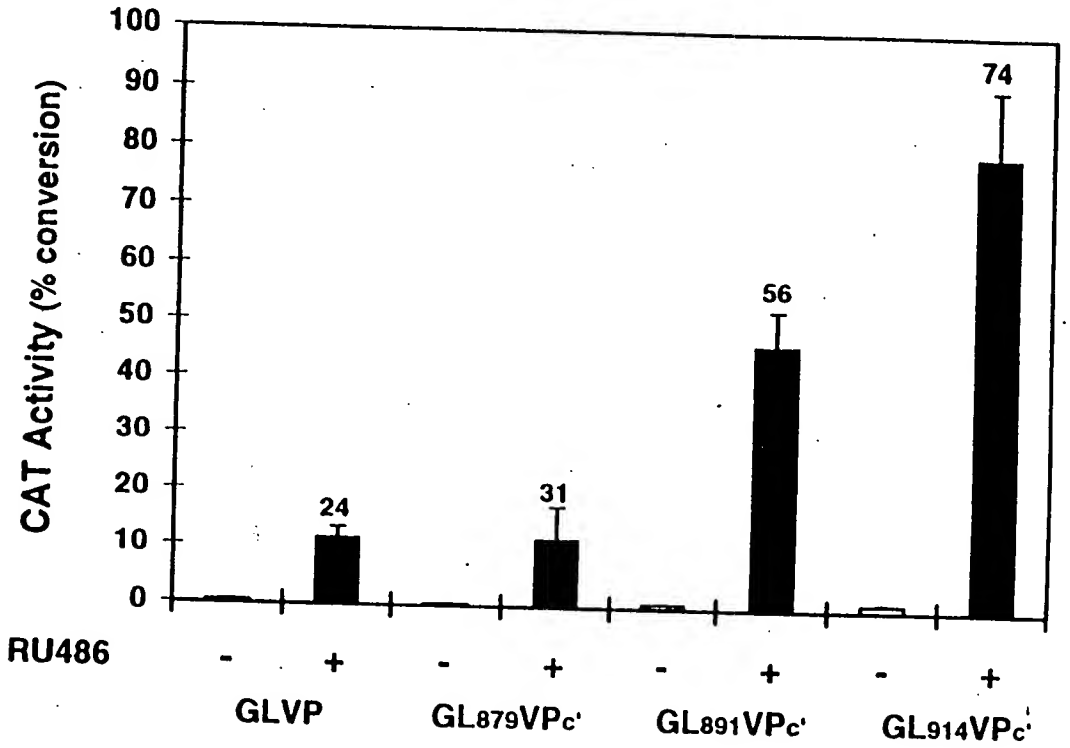

FIG. 19